# Inhibition of Intercellular Communication between Normal Human Embryonal Palatal Mesenchyme Cells by Teratogenic Glycol Ethers

## by Frank Welsch* and Donald B. Stedman*

Cell–cell communication, possibly through gap junctions, is a fundamental event for the differentiation of embryonal tissues. Chemical substances which can interfere with this process may be able to disrupt embryogenesis. We have examined the response of a normal diploid human embryonal palatal mesenchymal (HEPM) cell line to glycol ethers. These cells have gap junctions whose function in cell–cell communication was inhibited by a model teratogen. Potential HEPM donor cells (2000 to 4000) were pulse labeled with $^3$H-uridine (10 $\mu$Ci/mL; 3 hr) and then cocultured for 3 hr with 200,000 to 400,000 potential recipient cells in the absence or presence of 2-methoxyethanol (ME; 0.13–0.30 M) or 2-isopropoxyethanol (IPE; 0.05–0.1 M). Computer-assisted quantitative autoradiography was applied to assess the effects on metabolic cooperation. Although this phenomenon was inhibited by ME, the effect was probably not attributable to interference with gap junction-mediated transfer of labeled nucleotides but rather to the lack of formation of gap junctions resulting from cytotoxicity and poor physical contact between cells. The inhibition obtained with IPE was apparently not due to adverse effects on HEPM cells as judged by light microscopy and cell counts of recipients surrounding a given donor. The results suggest that HEPM cells are suitable to study disruption of cell–cell communication as a mechanism responsible for teratogenesis and offer the unique possibility to apply human embryonal cells. However, care has to be taken to assess potential cytotoxicity of xenobiotics, and further refinement of the criteria to detect adverse effects is required.

## Introduction

Direct cell–cell contact and transfer of chemical messenger molecules is believed to be a prerequisite for the differentiation of many embryonal systems. Although it is not clear whether such interactions involve preferably cell surface molecules or are mediated by the establishment of specialized junctions formed by cell membranes, there is experimental evidence for the existence in progressively developing systems of both modes of cell–cell interactions. Several investigators have proposed that a common surface formation of mammalian cells known as gap junction mediates cell–cell communication (which is also called intercellular communication) in developing embryos, and Lo has summarized most of the existing information and discussed its possible implications as well as the challenges for future experimental investigations (1,2). Cell interactions constitute a pivotal mechanism in differentiation and morphogenesis, and the geneti-

cally programmed building plan can only be realized if the cells can communicate with each other in a developmentally meaningful manner (3). Therefore, it appears reasonable to assume that interference with cell–cell communication may disrupt normal morphogenesis. Although gap junction-mediated transmission of developmental signals is an attractive possibility, no studies have yet been reported which have examined the effects of chemicals specifically inhibiting cell–cell communication in a developing system (4). There are considerable methodological complexities in performing such studies, because the most interesting observations in progressively developing mouse embryos have derived from electrical coupling measurements through microelectrode impalements of single cells and from the iontophoresis of fluorescein-labeled molecules into single cells (1,2).

Based on several commonalities between teratogenesis and carcinogenesis (5,6), it has recently been proposed that concepts developed from research in tumor promotion might be applicable to chemically induced teratogenesis (7). Experimental observations from cell culture studies of metabolic cooperation, a

*Chemical Industry Institute of Toxicology, P. O. Box 12137, Research Triangle Park, NC 27709.

specific form of cell–cell communication, have led Trosko and his associates to hypothesize that tumor-promoting chemicals may act by inhibiting metabolic cooperation. It was also proposed that the principle of disruption of cell–cell communication by xenobiotics may have relevance to an important mechanism of teratogenesis (7). When mechanisms recognized as being responsible for abnormal development are reviewed, it has been speculated repeatedly that chemical agents might alter membrane characteristics in the developing mammalian embryo and that this may lead to a failure of cell–cell interactions (3).

Cell cultures provide much simpler systems in which cell–cell communication can be studied, although in general the cells do not undergo progressive development with the notable exception of teratocarcinomas (1,4,8,9). For a first examination of the concept that among the structurally diverse chemical teratogens some may exert their adverse biological effects by inhibiting cell–cell communication, we used Chinese hamster V79 cells as Trosko and associates did in their tumor promoter studies, and we found that a variety of them, including several monoalkyl glycol ethers, inhibited metabolic cooperation (10).

In this communication we describe observations obtained with a cell line derived from a human abortus. We have recently established that these mesenchymal cells displayed metabolic cooperation in culture which appeared to be mediated by gap junctions and was inhibited by a chemical which interferes with cell–cell communication and is a potent tumor promoter and teratogen (11,12). The cells appeared to be particularly relevant for studies on the induction of abnormal development, because they derived from the palate region of a normal human embryo close to the time of palate closure (13) and contain high affinity glucocorticoid receptors. These recognition sites show pharmacologic specificity and may have relevance to cleft palate induction in the human embryo (14,15). In the present study we have exposed the human embryonal palatal mesenchyme (HEPM) cells to monoalkyl glycol ethers.

# Methods

## Cell Culture

The HEPM cells are diploid and presumably represent undifferentiated fibroblastlike cells from the palatal shelves. They were established in culture by the American Type Culture Collection (Rockville, MD, stock Nr. CRL 1486) (13). Some of the cells used in the present experiments were a gift (at passage 6) from Dr. Robert Pratt at NIEHS in the Research Triangle Park, in whose laboratory most of the published work with HEPM cells has been performed. Other cells were purchased from American Type Culture Collection at passage 3. The HEPM cells were maintained in culture

in Dulbecco's modified Eagle's minimum essential medium (DMEM; Gibco, Grand Island, NY) containing 10% bovine fetal serum (Hyclone Laboratories, Logan, UT) and 0.6 mg glutamine/mL. Stock cultures were grown in 150 $cm^2$ tissue culture flasks (Costar, Cambridge, MA) at 37°C in a humidified atmosphere containing 5% $CO_2$. The medium was changed every 3 to 4 days, and when the cells became confluent they were detached from their substratum with 0.05% trypsin and 0.02% EDTA (GIBCO) and subcultured.

## Detection of Metabolic Cooperation

The principles of the method developed by Pitts and Simms (16) were applied to perform autoradiographic measurements of metabolic cooperation between HEPM cells in culture. This assay is based on the transfer of labeled nucleotides, but not of RNA and other macromolecules, between prelabeled potential donor cells and unlabeled potential recipient cells during a coculture period at appropriate cell densities. Briefly, the assay conditions were as follows: between 2000 and 4000 donor HEPM cells were plated on 25 mm cover slips (Thermanox; Lab-Tek Division of Miles Laboratories, Naperville, IL) placed into 3.5 cm diameter Linbro multi-well culture plates (Flow Laboratories, McLean, VA) and cultured for 24 hr in 4 mL DMEM. These cells were then pulse labeled with $^3$H-urdine (10 μCi/mL; specific activity 20.7 Ci/mmole; New England Nuclear, Boston, MA). After removal of $^3$H-uridine and thorough washing the cover slips were transferred to fresh culture wells. Now 200,000 to 400,000 potential recipient cells were added in 4 mL DMEM with or without the addition of a chemical whose effect on metabolic cooperation was to be assessed. The coculture period with the prelabeled donor cells was 3 hr. At that time the cells were washed with phosphate-buffered saline (PBS) and fixed with 10% buffered formalin for 1 hr at 4°C. Unincorporated nucleotides were removed by washing twice for 5 min with 5% trichloroacetic acid (TCA) at 37°C. The cover slips were then mounted onto glass microscope slides and prepared for quantitative autoradiography by dipping them into NTB2 photographic emulsion (Kodak, Rochester, NY). Following 1 week of exposure at -20°C, the slides were developed in Kodak D-19 developer and Kodak fixative.

Initially the cells were stained with eosin and later on with Giemsa (Fisher Scientific). The experimental results were evaluated by counting the number of silver grains in the photoemulsion associated with an entire population of recipient cells in direct or indirect contact with a single donor cell whose grain content was not quantitated. Commonly 10 donor cells and their surrounding recipients were analyzed. An automated procedure developed by Dorman et al. (17) at this Institute was applied. The number of grains in one 78.5 $\mu m^2$ unit area in the cytoplasm of each recipient cell in direct or indirect contact with a donor cell was determined. Grain counts were performed with a Zeiss microscope attached

to a television camera and connected to an Artek Model 880 automatic grain counter (Artek Systems, Farmingdale, NY) which in turn was coupled to a Digital VAX/VMS 11/780 computer. Results were expressed both in number of grains per recipient cell and in number of recipient cells per donor. When the effect of treatment with a glycol ether was to be quantitated, the data were normalized so that the total grain count in an entire field of recipient cells surrounding each one of the 10 control donor cells selected for grain counting was set equal to 100%.

In the present series of experiments several factors were monitored to assess the effect of the presence of test chemicals on HEPM cells during the 3-hr coculture period. Among them were trypan blue exclusion and ³H-uridine incorporation studies using 2000 potential donor cells (pulse labeled with only 0.5 μCi/mL in order to reduce the labeling intensity and thus the grain numbers in order to make them manageable for the Artek automatic counting system) followed by quantitative autoradiography. In the case of 2-methoxyethanol electron microscopy was applied (see below).

## Preparation of Scanning Electron Micrographs (SEM)

About 200,000 cells were granted 24 hr attachment time onto cover slips as described and were then exposed to either 0.19 or 0.25 M 2-methoxyethanol for 3 hr as they were in the ³H-uridine nucleotide transfer assay. The HEPM cells were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2. They were dehydrated in a graded series of ethanol to 100% amyl acetate and critical point-dried in carbon dioxide. The specimens were attached to aluminum stubs with silver paste and coated with Au/Pd in a Hummer V sputter apparatus (Technics, San Jose, CA). The cells were viewed in an ETEC Autoscan scanning electron microscope (Perkin-Elmer E.T.B. Corporation, Hayward, CA). Images were recorded at 20 kV using Polaroid P/N 55 film.

## Metabolic Fate of ³H-Uridine in HEPM Cells

About 400,000 cells were plated as described above and granted 24 hr attachment time. Following pulse labeling, cells were washed and either harvested immediately or after further incubation in DMEM for 3 and 24 hr. Acid-soluble material was extracted twice with 5% TCA, and the cell sediment was washed prior to extraction of nucleic acids with 2 mL of 1 N NaOH for 1 hr at room temperature. An aliquot of this acid-insoluble, but alkali-soluble, material was neutralized with 1 N HCl. Radioactivity was then determined by liquid scintillation spectrometry.

Autoradiography was also performed on samples where coculture of 2000 donor cells with 200,000 recip-

ient cells was delayed for 24 hr after pulse labeling of the donors.

## Chemicals

The monoalkyl ethers of ethylene glycol examined in the present experiments were obtained from two sources. 2-Methoxyethanol (ME) was from Mallinckrodt

**Table 1. Incorporation of ³H-uridine into RNA of human embryonal palatal mesenchyme cells in the presence of glycol ethers.[a]**

| Treatment | Concentration, μL/mL medium | Grains per cell (mean ± SD) |
|---|---|---|
| Control | 0.0 | 35.90 ± 11.08 |
| 2-Methoxyethanol | 17.5 | 37.00 ± 12.08 |
| | 20.0 | 36.80 ± 9.30 |
| | 25.0 | 27.25 ± 7.09 |
| 2-Isopropoxyethanol | 7.5 | 37.40 ± 12.00 |
| | 15.0 | 28.90 ± 8.40 |

[a]About 2000 cover slip-attached cells were pulse labeled with 0.5 μCi ³H-uridine/mL for 3 hr in the presence of the glycol ethers whose concentrations are indicated. The cells were then prepared for quantitative autoradiography (see Methods for details). Values shown are the number of grains per donor and give the $\bar{x}$ ± SD for 10 potential donor cells at each concentration.
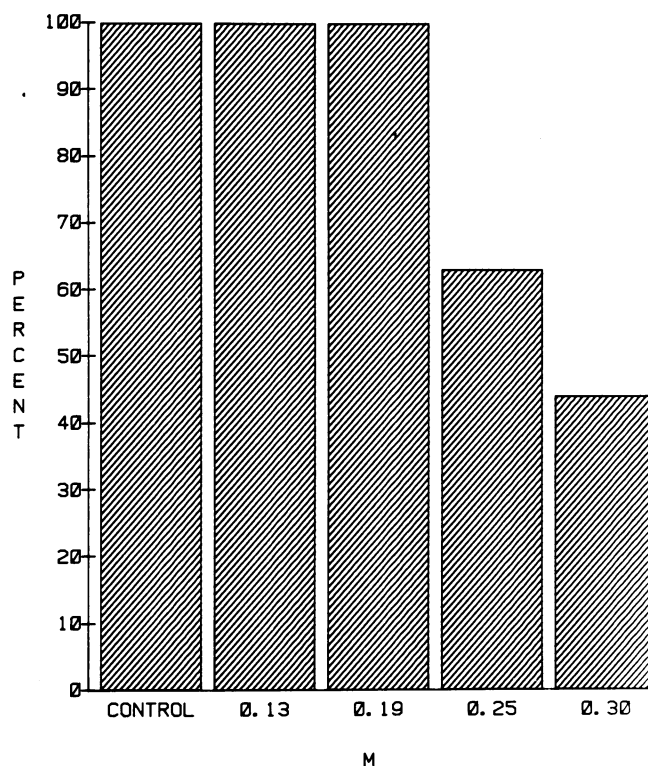


FIGURE 1. Effects of 2-methoxyethanol on ³H-uridine nucleotide transfer between HEPM cells. Eosin-stained preparations were evaluated by quantitative autoradiography. Total grain counts in entire fields of recipient cells surrounding 10 donor cells from untreated cultures were set to equal 100%, and 2-methoxyethanol effects were calculated relative to this control value.
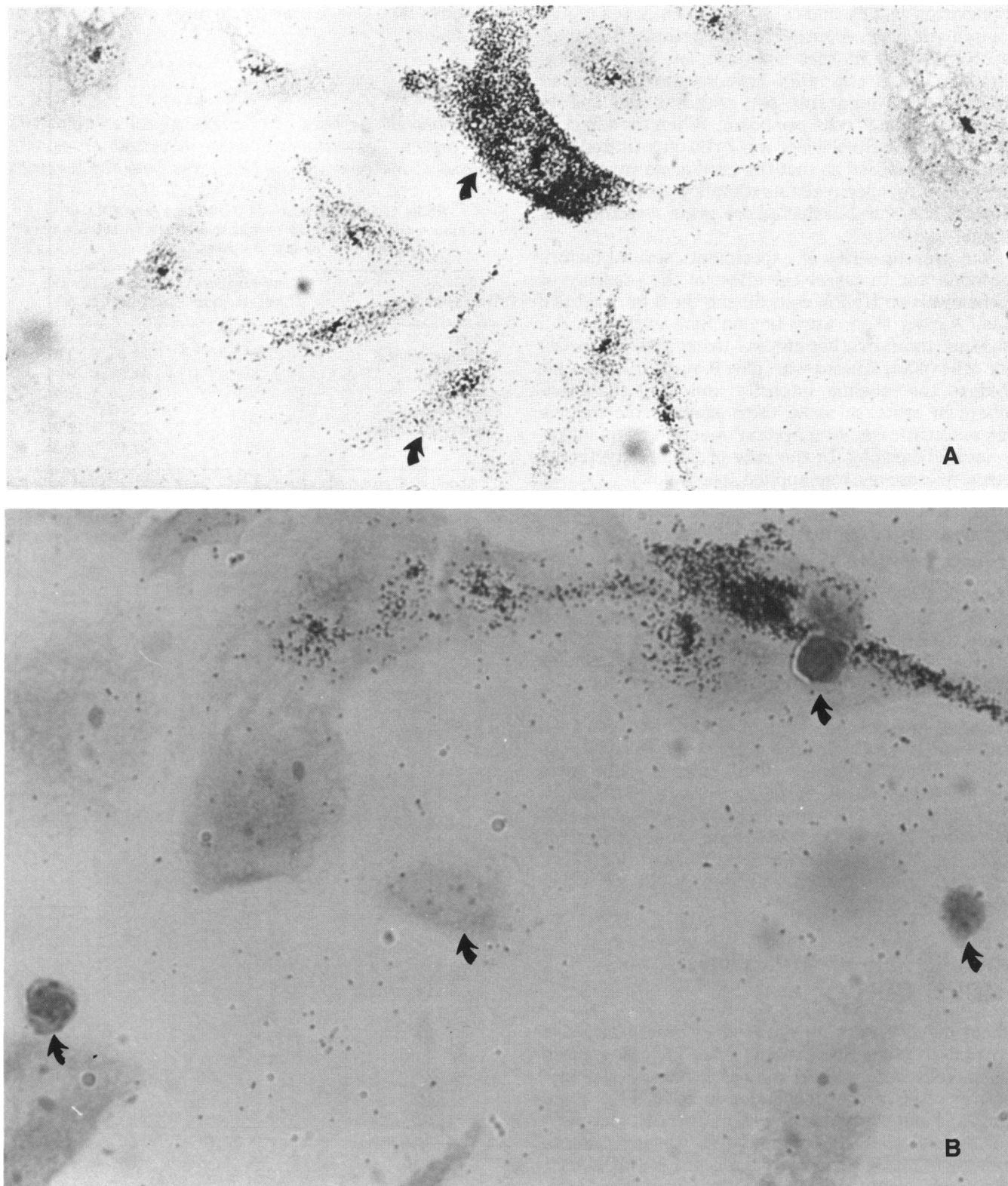
FIGURE 2.   Effects of 2-methoxyethanol on HEPM cells. This photomicrograph depicts the appearance of a Giemsa-stained preparation: (A) control culture shows a heavily labeled, irregularly shaped donor cell surrounded by cells with morphological characteristics typical for mesenchymal cells with irregular shapes and numerous processes (arrows), magnification ×200; (B) cells exposed to 0.25 M 2-methoxyethanol for 3 hr; rounding of the cells is noticeable among intensely stained cells (arrows), and there appear to be empty spaces between cells rather than cell–cell contacts as expected at this cell density and visible in (A); magnification ×200.

(St. Louis, MO) and 2-isopropoxyethanol (IPE) was from Fluka (Hauppauge, NY).

## Results and Discussion

Experiments reported elsewhere in part (11) and to be published elsewhere (12) have established that $^3$H-uridine-associated radioactivity was transferred via gap junctions between pulse-labeled potential donor cells and unlabeled potential recipient cells during coculture. Further analyses revealed that the material exchanged was of low molecular weight, compatible with nucleotides, and that RNA could not be passed between cells. This conclusion was based on the observation that radioactivity was no longer transferred between cells if coculture with potential recipient cells was delayed for 24 hr after pulse labeling. At that time more than 90% of the label had become acid-insoluble and was presumably incorporated into RNA. The glycol ethers whose effect on the transfer of labeled nucleotides was studied in the present experiments were selected because ME (18,19) caused abnormal development in laboratory animals. All monoalkyl derivatives which were examined in V79 cell cultures inhibited metabolic cooperation, and IPE which has apparently not yet been tested for its teratogenic potential in vivo was the most efficacious one (10). The selection of the concentrations to be examined in the present experiments was based on experience with V79 cells, although the criteria used for cytotoxicity assessments in that cell line were not necessarily applicable to HEPM cells because of the different nature of the cells and the much longer exposure time to chemicals which lasted for 3 days in V79 cells. When trypan blue exclusion from HEPM cells following 3 hr of exposure to the glycol ethers was used as a criterion, there was no evidence of cytotoxicity. Pulse labeling of potential donor cells in the presence of various concentrations of ME or IPE caused no significant inhibition of incorporation of $^3$H-uridine into RNA (Table 1).

Quantitative autoradiographic analysis of eosin-stained cell cultures suggested significant, concentration-related reductions in labeled nucleotide transfer when the cells were exposed to 2-ME during coculture. A solvent control was not required because of the excellent water solubility of the glycol ethers. Grain counts in entire fields of recipient cells surrounding 10 donor cells and making direct or indirect contact with the donors suggested good correlation between the extent of nucleotide transfer inhibition and ME concentration. When the total grain number in all control recipient cells was set to be 100% and all ME-treated cocultures were expressed relative to this value, 0.25 M reduced grain transfer by 37% and 0.30 M by 56% (Fig. 1). These effects were attributed to an inhibition of metabolic cooperation, presumably mediated by gap junctions, caused by ME. This interpretation of the observations was made by extrapolation from our quantitative morphometric analysis of HEPM

gap junctions in response to the teratogen 12-O-tetradecanoyl-phorbol-13-acetate (12).

In later experiments the autoradiograms were stained with Giemsa because eosin did not make cell contures well visible. However, the silver grain of the photoemulsion allowed easy distinction between intensely labeled donor cells, recipient cells in direct contact with the donors and those that made only indirect contact through other recipient cells and thus contained fewer grains per cell. Quantitative autoradiography and the interpretation of the experimental observations were initially based on eosin stained cell cultures. Giemsa, however, revealed more details of cell membranes and other cell fine structure at the level of light microscopy than eosin did and suggested that ME had adverse effects on the morphology of the HEPM cells. Some of the cells looked as if they had rounded off and were staining much more intensely than cells in control autoradiograms. Furthermore, the cell density appeared to be reduced because many cells were not flattened out on the substratum and took less space than untreated cells (Fig. 2). This impression was further supported by a reduction in the number of recipient cells visible per field surrounding one donor cell (Fig. 3).

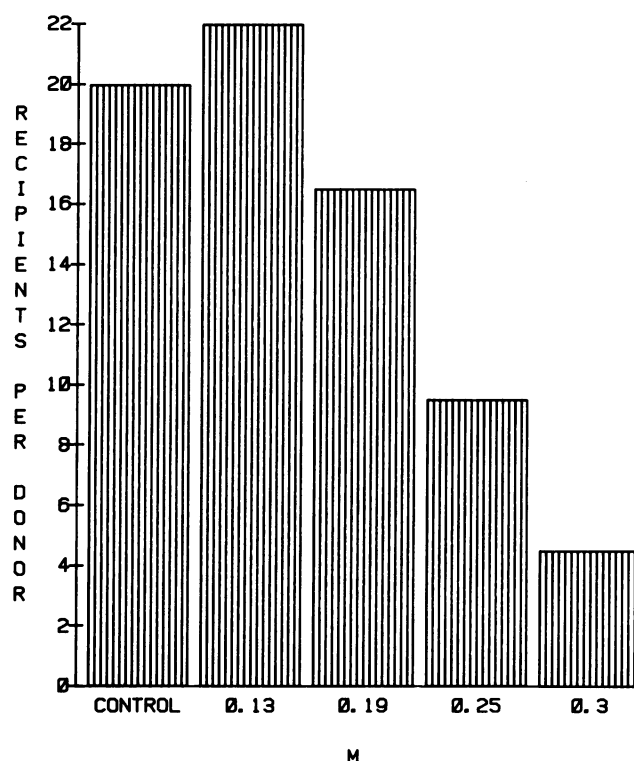Additional confirmation for deleterious effects of ME



FIGURE 3. Effects of 2-methoxyethanol on the number of potential recipient cells surrounding potential donor cells. Giemsa-stained autoradiograms were used to quantitate both the total grain and the number of recipient cells in a given field around a donor cell which is indicated on the ordinate. The concentrations of 2-methoxyethanol are shown on the abscissa and are equal to those shown in Fig. 1.
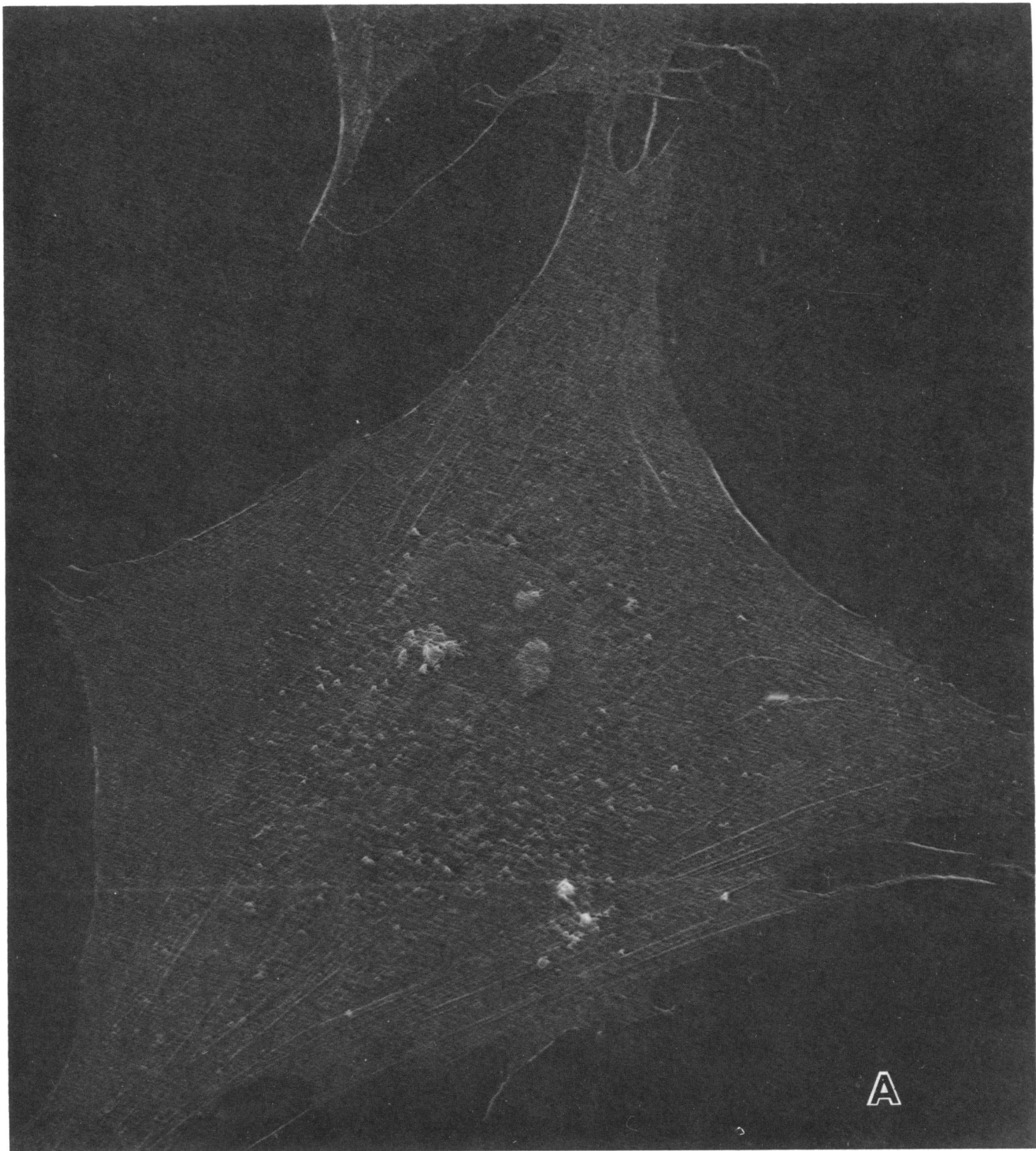
FIGURE 4.    Scanning electron micrographs of HEPM cells cultured under control conditions or exposed to 0.19 M 2-methoxyethanol for 3 hr: (A)
control culture with a cell that has the morphological characteristics typical for mesenchymal (prefibroblastic) cells in culture and makes
contact through cytoplasmic processes with a neighboring cell, magnification ×600; (B) 0.19 M 2-methoxyethanol for 3 hr yields rounded
cell with extensive bleb formation, magnification ×3000.
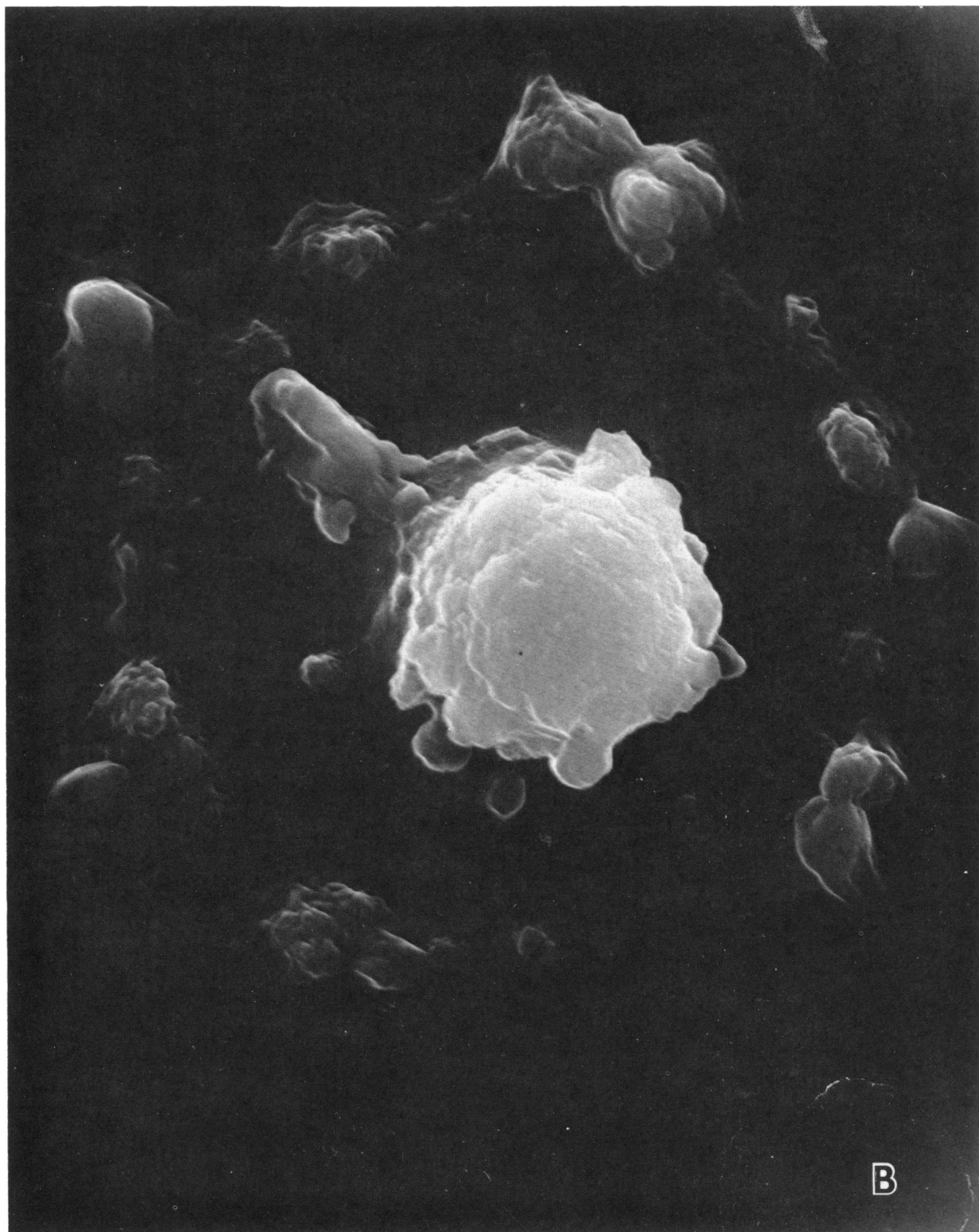
on HEPM cells was subsequently obtained by SEM of cells exposed to 0.19 M and 0.25 M ME for 3 hr analogous to the coculture experiments (Fig. 4). It was observed that 0.19 M caused rounding of many cells and disappearance of morphological features typical for these mesenchymal (prefibroblastic) cells grown under control conditions in DMEM (Fig. 4 A). The loss of cell–cell contacts was quite obvious in cells exposed to ME. Another notable feature was extensive bleb formation resulting from exposure to 0.19 M ME (Fig. 4B). These adverse effects on the morphology of the cells were even more pronounced following 0.25 M (not shown). Therefore, it appeared as if the reduction of metabolic cooperation observed in eosin-stained autoradiograms was not the result of an inhibition of gap junction-mediated transfer of $^3$H-uridine nucleotides. Rather, the morphological effects suggested a lack of establishment of gap junctions which are formed at sites of cell–cell contacts (4), because many cells were rounding off and no longer making physical contact with one another and with the $^3$H-uridine-labeled potential donor cells. These actions of ME on HEPM cell integrity were quite different from those produced in Chinese hamster V79 cells. Following exposure to comparable concentrations of ME for 3 days, metabolic cooperation was significantly inhibited in V79 cells without interference with plating efficiency (10). There

appeared to be no adverse effects on the morphology of V79 cells when exposed to ME for 3 hr and examined by SEM (unpublished observations).

The effect on metabolic cooperation between HEPM cells subsequent to IPE exposure was quite different from that with ME. Concentrations ranging from 0.052 via 0.070 and 0.090 to 0.100 M caused marked reductions of $^3$H-uridine transfer (Fig. 5A). In Giemsa stained cultures there was no evidence of IPE-induced damage to the morphology of the HEPM cells when judged by light microscopy, except for beginning signs of some change in cell membrane-staining characteristics at 0.1 M IPE. There was no evidence at this level of optical resolution that IPE had caused changes anywhere comparable to those illustrated following ME. This was reflected in the unchanged number of potential recipient cells in a given field surrounding a donor over the entire concentration range of IPE (Fig. 5 B) in contrast to ME where damage to the cell morphology was also reflected in a decline in the number of recipients per donor (Fig. 3). The observations in HEPM cells agreed with our conclusions drawn from experiments with V79 cells. With that line IPE was considerably more potent than ME in inhibiting metabolic cooperation as witnessed by significant effects at lower concentrations. Furthermore, IPE in V79 cells was much more efficacious than ME (10).
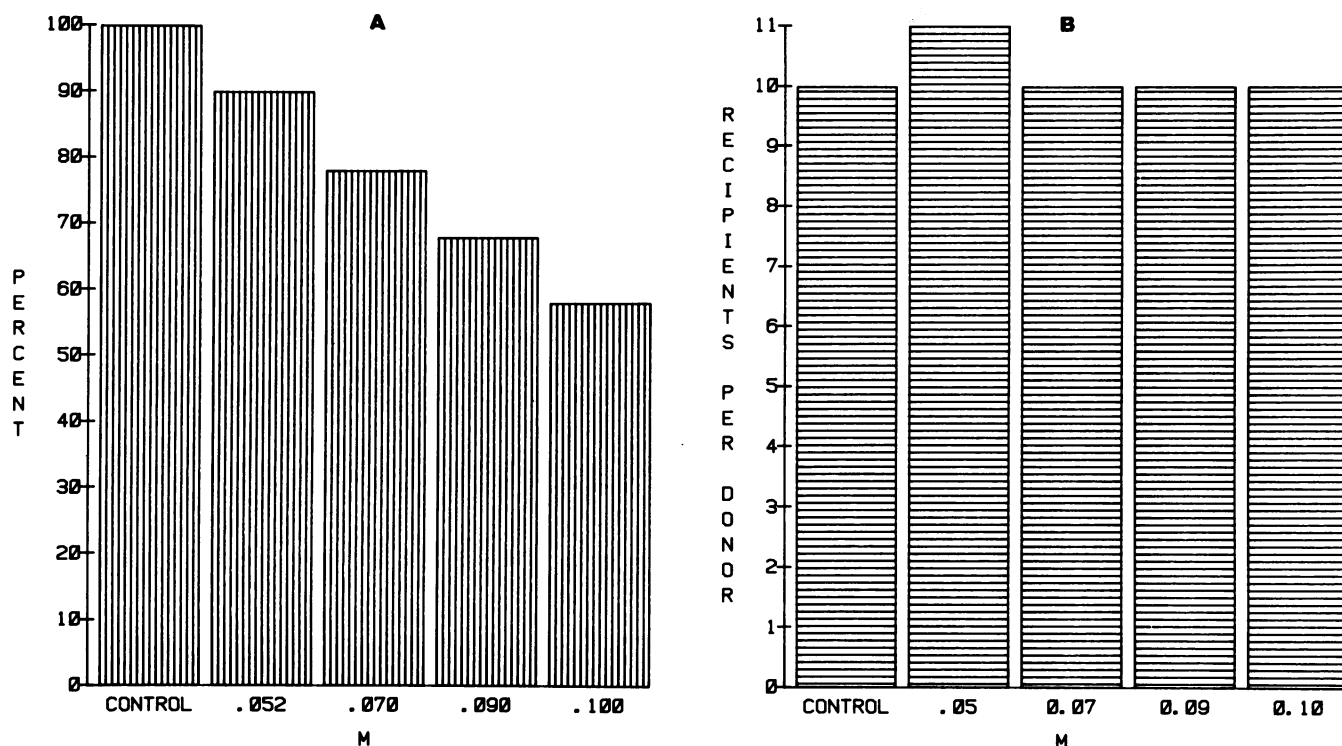


FIGURE 5.    Effects of 2-isopropoxyethanol on $^3$H-uridine nucleotide transfer between HEPM cells. Giemsa stained autoradiograms were used to quantitate both the total grain and the number of recipient cells in a given field around a single donor cell. (A) Values obtained by total grain counts in entire fields of recipient cells surrounding 10 donor cells in control cultures were set to equal 100%. Results from cells exposed to various concentrations of 2-isopropoxyethanol as indicated on the abscissa were related to this value and expressed on the ordinate as percent of control. (B) The number of recipient cells per donor is expressed on the ordinate and shows no difference from control autoradiograms over the range of 2-isopropoxyethanol concentrations which is given in the abscissa.

It is difficult to compare doses of glycol ethers administered to pregnant animals with those used in the present cell culture studies. Nevertheless, it may be informative to mention that ME induced deviations in structural development after oral doses of 1 to 3 mmoles/kg for 8 consecutive days during mouse organogenesis (18). Dermal application of 40 mmoles 2-ethoxyethanol/kg or more for 10 days to pregnant rats caused malformations (20). Furthermore, comparisons for specific glycol ethers are hampered by the different routes of administration of the compounds used in completed or still ongoing in vivo testing.

Our observations reported here underline the need for monitoring the effects of chemicals on cell morphology. It has been postulated, based on metabolic cooperation experiments with a variety of cell lines, that cell shape could provide a means by which the extent of cell–cell communication in intact tissues of embryos might be changed. Alterations in shape may alter the number of gap junction channels formed between various cell types (1). Conceptually, it appears appealing to assume that changes in the number of junctions established between cells could determine the rate of exchange of materials between cells. Equally attractive is the hypothesis that the extent of intercellular communication might be quantitatively regulated by the closing of existing channels between cells at the sites of cell–cell contacts which were previously open and functioning. Some experimental evidence obtained in cultured mouse embryos in support of this assumption exists (1). The present observations indicate that the inhibition of labeled nucleotide transfer resulting from ME exposure may be in part attributable to alterations in cell shape induced by the chemical. Further biochemical and ultrastructural criteria are needed to better define the consequences of ME exposure on cell viability.

The inhibition of metabolic cooperation between HEPM cells by tetradecanoyl phorbol acetate was correlated to ultrastructural changes in gap junction appearance compatible with the idea of chemically induced disappearance of channels (12). While comparable freeze fracture electron microscopy analyses have not yet been performed in cells exposed to IPE, the light microscopic morphology suggested that the inhibitory effect of IPE on $^3$H-nucleotide transfer was not the result of alterations in cell shape comparable to those induced by ME.

The effects of chemical teratogens which we have so far observed in both V79 hamster (10) and in HEPM cells (11, 12) encourage further investigations in cell culture or in a progressively developing culture system (1,2) of the phenomenon of cell–cell communication, its disruption by xenobiotics and the possible relevance of the effect to congenital malformations.

## REFERENCES

1. Lo, C. W. Gap junctions in development. In: Development of Mammals, Vol. 4 (M. H. Johnson, Ed.), Elsevier, New York, 1980, pp. 39–80.
2. Lo, C. W. Gap junctional communication compartments and development. In: The Functional Integration of Cells in Animal Tissues (J. D. Pitts and M. E. Finbow, Eds.), Cambridge Univ. Press, Cambridge, 1982, pp. 167–179.
3. Saxen, L. Abnormal cellular and tissue interactions. In: Handbook of Teratology, Vol. 2 (J. G. Wilson and F. C. Fraser, Eds.), Plenum Press, New York, 1977, pp. 171–197.
4. Hooper, M. L. Metabolic cooperation between mammalian cells in culture. Biochim. Biophys. Acta 651: 85–103 (1982).
5. Miller, R. W. Relationship between human teratogens and carcinogens. J. Natl. Cancer Inst. 58: 471–474 (1977).
6. Bolande, R. P. Teratogenesis and oncogenesis. In: Handbook of Teratology, Vol. 2 (J. G. Wilson and F. C. Fraser, Eds.), Plenum Press, New York, 1977, pp. 293–325.
7. Trosko, J. E., Chang, C. C., and Netzloff, M. The role of inhibited cell–cell communication in teratogenesis. Teratogen. Carcinogen. Mutagen. 2: 31–45 (1982).
8. Martin, G. R. Teratocarcinomas and mammalian embryogenesis. Science 209: 768–776 (1980).
9. Strickland, S., Smith, K. K., and Marotti, K. R. Hormonal induction of differentiation in teratocarcinoma stem cells: generation of parietal endoderm by retinoic acid and dibutyryl cAMP. Cell 21: 347–355 (1980).
10. Welsch, F., and Stedman, D. B. Inhibition of metabolic cooperation between Chinese hamster V79 cells by structurally diverse teratogens. Teratogen. Carcinogen. Mutagen, in press.
11. Welsch, F., and Stedman, D. B. Transfer of $^3$H-uridine nucleotides between human embryonal palatal mesenchyme cells and its inhibition by teratogens. Pharmacologist 25: 191A (1983).
12. Welsch, F., Stedman, D. B., and Carson, J. Transfer of nucleotides between human embryonal cells is mediated by gap junctions and inhibited by a teratogen. Submitted.
13. Yoneda, T., and Pratt, R. M. Mesenchymal cells from the human embryonic palate are highly responsive to epidermal growth factor. Science 213: 563–565 (1981).
14. Yoneda, T., and Pratt, R. M. Glucocorticoid receptors in palatal mesenchymal cells from the human embryo: relevance to human cleft palate formation. J. Craniofacial Gen. Develop. Biol. 1: 411–423 (1981).
15. Pratt, R. M. Mechanisms of chemically-induced cleft palate. Trends Pharmacol. Sci. 4: 160–162 (1983).
16. Pitts, J. D., and Simms, J. W. Permeability of junctions between animal cells. Intercellular transfer of nucleotides but not of macromolecules. Exptl. Cell Res. 104: 153–163 (1977).
17. Dorman, H. B., Butterworth, B. E., and Boreiko, C. J. Role of intercellular communication in the promotion of C3H/10T 1/2 cell transformation. Carcinogenesis 4: 1109–1115 (1983).
18. Nagano, K., Nakayama, E., Oobayashi, H., Yamada, T., Adachi, M., Nishizawa, T., Ozawa, M., Nakaichi, M., Okuda, M., Minami, K., and Yamazaki, K. Embryotoxic effects of ethylene glycol monomethyl ether. Toxicology 20: 335–343 (1981).
19. Anonymous. U. S. Department of Health and Human Services (NIOSH) Publication No. 83-112, Current Intelligence Bulletin 39 (May 2, 1983).
20. Hardin, B. D., Niemeier, R. W., Smith, R. J., Kuczuk, M. M., Mathinos, P. R., and Weaver, T. F. Teratogenicity of 2-ethoxyethanol by dermal application. Drug Chem. Toxicol. 5: 277–294 (1982).